ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 12, Issue 9, 2019

Online - 2455-3891
Print - 0974-2441

Research Article

EFFECTS OF *CINNAMOMUM ZEYLANICUM* BARK EXTRACT ON NOCICEPTION AND ANXIETY LIKE BEHAVIOR IN MICE

SEEMA JAIN[1], SPARSH GUPTA[2]*

[1]Department of Pharmacology, University College of Medical Sciences and Guru Teg Bahadur Hospital, New Delhi, India. [2]Department of Pharmacology, Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi, India. Email: gsparsh117@gmail.com

Received: 11 December 2018, Revised and Accepted: 25 July 2019

ABSTRACT

Objectives: The aim of the study was to assess the effect of the extract of *Cinnamomum zeylanicum* (CZ) bark in the experimental models of pain and anxiety-like behavior in mice.

Methods: The extract of CZ bark was administered at the doses of 100, 200, and 400 mg/kg, per orally (p.o) and morphine used as a positive control for pain models, was administered at the dose of 5 mg/kg, intraperitoneally (i.p.). Antinociceptive activity was evaluated using three experimental animal models of pain, namely, tail flick, hot plate, and formalin test. Elevated plus maze test was used to assess the effect on anxiety-like behavior. Rotarod apparatus and actophotometer were used to test muscle coordination and locomotor activity, respectively.

Results: Administration of CZ bark extract in the dose of 200 and 400 mg/kg showed significantly increased in the tail-flick latency and latency to reaction time in hot plate test as compared to the control group. In the first phase (0–5 min) of the formalin test, a significant reduction in the pain response was found in CZ (200 and 400 mg/kg) and morphine-treated groups, however during the second phase (30–35 min) significant reduction in formalin-induced pain response was observed in 100, 200, and 400 mg/kg CZ extract-treated group when compared to control group. CZ extract administration at 200 and 400 mg/kg dose caused a significant increase in the percentage of time spent in open arms in the elevated plus maze as compared to the control group.

Conclusion: Results suggest that CZ bark extract possesses the antinociceptive activity and modulates anxiety-like behavior.

Keywords: *Cinnamomum zeylanicum,* Tail flick, Formalin test, Hot plate, Elevated plus maze.

 DOI: http://dx.doi.org/10.22159/ajpcr.2019.v12i9.31287

INTRODUCTION

The bark of *Cinnamomum zeylanicum* (CZ) or *Cinnamomum verum* plant is commonly added as spices and flavoring agent in the food products. In addition, oils/extracts derived from different parts of this plant are also used in Indian folk medicine for various disorders since ancient period [1]. *Cinnamomum zeylanicum* belongs to the family Lauraceae and is commonly known as Ceylon or True cinnamon. Various parts of the cinnamon plant have been used since old times for the treatment of various ailments such as flatulent dyspepsia, anorexia, toothaches, cough, inflammatory conditions, and intestinal colic. [2-4]. Cinnamon has also been reported to have antibacterial [5], antifungal [6], antipyretic [7], antidiabetic [8], hypolipidemic [9], antioxidant [10], and uterine stimulant activities [11]. The experimental and clinical studies have also proven its efficacy in the treatment of type II diabetes and insulin resistance which is attributed due to the presence of methyl-hydroxy-chalcone polymer compound in the cinnamon [8].

The beneficial effects produced by cinnamon are attributed to the presence of several bioactive compounds such as cinnamaldehyde, eugenol, trans-cinnamic acid, phenolic compounds, catechins, terpenoids gum, mucilage, resin, starch, and sugar in the cinnamon [12,13]. Cinnamaldehyde, a phenolic compound is identified as the main phytochemicals found in cinnamon bark and is responsible for most of the biological effects of cinnamon. Various pharmacological properties such as antifungal, anticancer, antimutagenic, anti-inflammatory, neuroprotective, and antioxidant effects are considered mainly due to the presence of cinnamaldehyde and eugenol in cinnamon [14,15]. It is well established that extracts and oils obtained from cinnamon have strong free radical scavenging or antioxidant activity due to the presence of flavonoids, polyphenolic, and phenolic compounds in the cinnamon [10,16].

Data from the literature have also reported the anti-inflammatory effect of CZ. In one study, 2'- hydroxy cinnamaldehyde derived from the bark of *Cinnamomum cassia* has been found to inhibit the transcriptional activity of nuclear factor (NF) -kB and production of nitric oxide (NO) in lipopolysaccharide-induced inflammation models [17]. A recent study has shown that trans-cinnamaldehyde obtained from *Cinnamomum cassia* bark suppressed activation of microglia and the neuroinflammatory process by inhibiting the production of NO and expression of inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), and interleukin-1β (IL-1β) [18]. Furthermore, administration of ethanol extract of CZ has been found to inhibit the intracellular release of tumor necrosis factor-alpha (TNF-α) and TNF-α gene expression, suggesting its anti-inflammatory property [19].

A study performed in our laboratory has demonstrated that administration of CZ extract also improved cognitive performance in scopolamine treated animals [13]. Although the analgesic activity of CZ has been demonstrated in some studies [20-23] using the hot plate and acetic- induced writhing tests, we could not find any published data using tail flick and formalin-induced pain models for testing of the antinociceptive effect of cinnamon. Therefore, the present study was carried out to investigate the effect of CZ extract in animal models of pain (tail flick, hot plate, and formalin test) and anxiety (elevated plus maze). Besides, the effect on locomotor activity and muscle coordination was also assessed using actophotometer and rotarod apparatus, respectively.

MATERIALS AND METHODS

Animals

Swiss male albino mice weighing 24–30 g were used in the study. Animals were procured from the Central Animal House, University College of Medical Sciences, University of Delhi, Delhi. Animals were

housed in groups of six mice per cage with a natural light/dark cycle and provided with free access to pellet diet and water. Procedures adopted during experiments on animals and their care were conducted in accordance with the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals, India, and were approved by Institutional Animal Ethics Committee, University College of Medical Sciences, University of Delhi, Delhi.

Drug and dosing schedule

The hydroalcoholic extract of the bark of CZ (Batch No: DCP 23210) was supplied by M/S Tapovan Ayurved Sadan, New Delhi (Member: Central Institute of Medicinal and Aromatic Plants, Lucknow, India). Preliminary phytochemical screening of the extract was carried out for the detection of phytoconstituents using reference chemical tests [24]. Further, the extract was also analyzed by gas chromatography-mass spectrometry (GCMS-QP2010 Plus, Shimadzu). Rtx®-5MS (60 m´ 0.25 mm I.D. df=0.25 µm) film thickness column was used for analysis. The peaks were detected on the total ion chromatogram and were identified using the Wiley 8 and NIST05 mass spectral library. For the purpose of study extract of CZ bark was suspended in distilled water using carboxymethylcellulose (CMC) and administered per orally (p.o.) in the doses of 100, 200, and 400 mg/kg while morphine (5 mg/kg, i.p.) and diazepam (1 mg/kg, i.p.) were used as positive control for assessment of nociceptive response and anxiety, respectively.

Experimental designs for assessment of antinociceptive activity

Tail flick test

Tail flick test that represents the thermal model of pain was carried out using tail-flick analgesiometer (Ugo Basile, Italy) consisting of an infrared radiant light source. To measure tail flick response, each animal was gently held with, on the one hand, and it was positioned on tail flick unit so that lower part of the tail of the animal is exposed to radiant heat. To circumvent damage of tail due to constant heat, a cutoff time of 15 s was set in the instrument so that the timer and radiant heat source get automatically discontinue after 15 s. The response or tail flick latency (TFL) was measured as time (s) taken by each animal to flick its tail. Total of five groups comprising six mice in each group was used for the test. Group I acted as the control group and was administered normal saline while II, III, and IV group received the extract of CZ bark in the dose of 100, 200, and 400mg/kg, p.o, respectively. Group V was treated with morphine at a dose of 5 mg/kg, i.p. TFL in each group was measured before (baseline) and after 30, 60, 90, and 120 min after administration of testing drugs/saline [25].

Hot plate test

Five different groups of mice were used. Group I received normal saline and worked as the control group, animals of Groups II, III, and IV received CZ bark extract orally in the doses of 100, 200, and 400 mg/kg, respectively, while animals of Group V received morphine intraperitoneally in the dose of 5 mg/kg. To assess antinociceptive activity in different groups, each animal was placed on a hot plate, set at a temperature of 55°±0.5°C. The reaction time to the thermal stimulus was taken before the administration of drugs (baseline) and then at 30, 60, 90, and 120 min after CZ extract and morphine administration. The reaction time (latency) to thermal stimuli was measured as the time taken by the animal for licking of paws or jumping response and was noted for each animal in all the groups. The cutoff time was taken as 30 s to protect the animal from tissue damage [26].

Formalin test

Nociception in animals was induced by injecting formalin in the paw of the animal according to the method described by Abbott *et al*. [27]. 0.05 ml of 1% formalin in distilled water was injected into the dorsal surface of the right hind paw. The animals were immediately placed into the testing chamber. Following formalin injection, nociceptive response was recorded in two phases. The first phase represents neurogenic pain response and lasts from 0 to 5 min immediately after the injection of formalin. The second phase represents inflammatory pain response and occurs 30–35 min after formalin injection. The nociceptive response was measured during both the phases as duration (in seconds) spent by the animal in licking or biting of injected hind paw. Normal saline, CZ extracts (100, 200, and 400 mg/kg, p.o.) were administered 1 h before formalin injection in Groups I, II, III, and IV, respectively, while morphine (5 mg/kg, i.p.) was given 30 min before formalin injection in Group V.

Assessment of locomotor activity

Locomotor activity was evaluated by means of actophotometer apparatus (INCO, Ambala, India). The apparatus contains a square arena and operates on photoelectric cells connected in circuit to the counter. As the beam of light falling on photocell is cut off by the animal, a count is recorded as a measure of locomotor activity. The locomotor activity was recorded for a period of 5 min, 1 h after administration of CMC in distilled water in Group I and CZ bark extract at 100, 200, and 400 mg/kg dose in Groups II, III, and IV, respectively [28,29].

Assessment of motor coordination (Rotarod test)

This test was used for evaluation of neuromuscular coordination in animals. It was performed using horizontal rotation rod device (INCO, Ambala, India). Before administration of extract/vehicle, fall off time from the rolling rod was recorded for each animal. Then, animals were divided into four groups of six mice per group. Group I received CMC in distilled water and served as control. While Groups II, III, and IV received CZ extract in the dose of 100, 200, and 400mg/kg, p.o., respectively. Animals were again placed on the rods 1 h after the treatment and their fall off time [seconds] from the rotating rod was noted in each group. [30,31].

Elevated plus maze test

This test has been widely used to measure anxiety in rodents. The maze was made of wood painted gray and contained a central platform (8 × 8 cm) from which radiate four symmetrical arms (16 × 5 × 10 cm) and elevated to a height of 25 cm. Total of five groups of animals were used. Group I received CMC in distilled water (vehicle-treated group). Groups II, III, and IV were given CZ extract at the dose of 100, 200, and 400 mg/kg, p.o., respectively, while Group V received diazepam (1 mg/kg, i.p.) as the positive control. One hour after administration of vehicle and CZ extract and 30 min after administration of diazepam each mouse was placed in the center of the maze, facing toward open arm and the number of entries in open arms, enclosed arms and time spent in enclosed and open arms were recorded for a period of 5 min [32].

Statistical analysis

Data are expressed as the Mean±S.E.M. Analysis of variance (ANOVA) followed by Bonferroni *post hoc* test was applied for the analysis of results. Results were considered significant at $p<0.05$.

RESULTS

On phytochemical screening of extract presence of flavonoids, tannins, saponins, phenolic compounds, and sugars was detected in the extract. Analysis results of the extract on GC-MS showed 42 peaks and the presence of many active principles (Fig. 1).

Effect on tail flick test

Administration of CZ bark extract at the doses of 200 and 400 mg/kg caused a significant prolongation of mean TFL at 60 min ($p<0.05$ and $p<0.001$, respectively) and 90 min ($p<0.05$ and $p<0.01$, respectively) when compared to control group. Administration of morphine (5 mg/kg) also caused a significant prolongation of latency as compared to control group at 30 min ($p<0.01$), 60 min ($p<0.001$), and 90 min ($p<0.001$) (Fig. 2).

Effect on hot plate test

Pretreatment of CZ bark extract in 200 mg/kg showed significant prolongation of latency to reaction time at 60 and 90 min ($p<0.05$) as compared to the control group. Similar prolongation of latency was

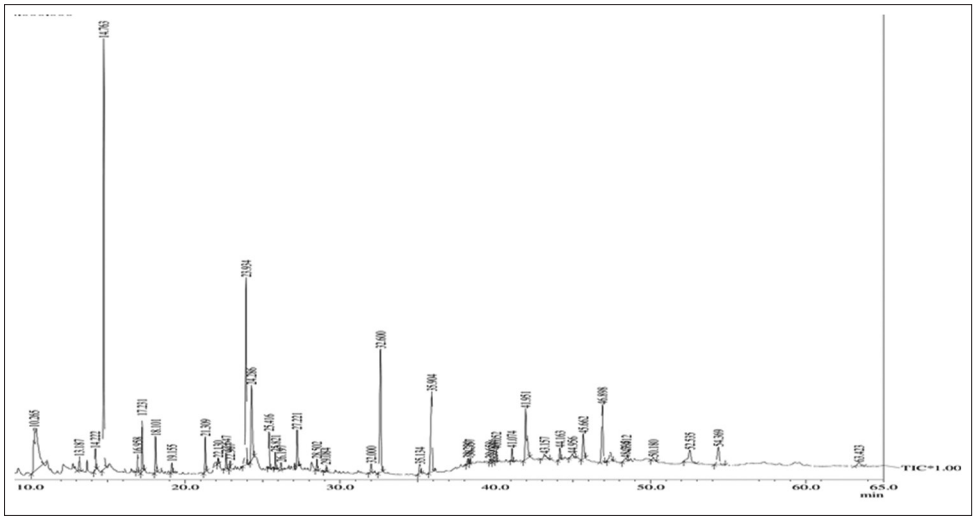

Fig. 1: Gas chromatography-mass spectrometry chromatogram of the extract of *Cinnamomum zeylanicum* bark

observed with 400 mg/kg dose of extract at 60 and 90 min ($p<0.01$) when compared to the control group. Administration of morphine as positive control caused significant ($p<0.001$) increase in hot plate latency at 30, 60, and 90 min (Fig. 3).

Effect on formalin-induced pain response

Administration of CZ extract at the dose of 200 and 400 mg/kg during the first phase (0–5 min) showed significant ($p<0.05$ and $p<0.001$, respectively) decrease in the duration of formalin-induced pain response as compared to control group. However, in the second phase (30–35 min) the duration of response found to decrease in all three (100, 200, and 400 mg/kg) tested doses of CZ extract-treated group ($p<0.01$, $p<0.001$, and $p<0.001$, respectively) when compared to control group. Administration of morphine inhibited pain responses in both the first and second phase ($p<0.001$) (Fig. 4).

Effect on locomotor activity and motor coordination

No significant increase or decrease in locomotor activity was observed after administration of extract (100, 200. and 400 mg/kg, p.o.) in treated mice as compared to control mice. The CZ extract in all the three doses did not produce any significant effect on the motor coordination as there was no significant difference in the time of fall between controls and extract-treated groups (Table 1).

Effect on anxiety-like behavior

Administration of CZ bark extract in 200 mg/kg ($p<0.05$) and 400 mg/kg ($p<0.01$) dose showed a significant increase in the percentage of time spent in open arms when compared with the control group. In diazepam (1 mg/kg, i.p.) treated group also a significant increase in the percentage of open arm entries ($p<0.001$) and time spent in open arm ($p<0.001$) was observed as compared to the control group (Fig. 5a and b).

DISCUSSION

The present study demonstrates the antinociceptive effects of hydroalcoholic extract of CZ bark on three animal models of acute pain. In addition, effect of the extract was also investigated on anxiety like behavior, locomotor activity, and muscle coordination in mice. The results clearly showed that oral administration of hydroalcoholic extract CZ bark significantly prolonged the latency of nociceptive response in tested animal models of pain when compared with saline-treated animals.

True cinnamon (family Lauraceae) is one of the oldest herbal medicines known mentioned in Chinese texts as early as 4000 years ago [33]. The aromatic bark obtained from the cinnamon tree is used worldwide

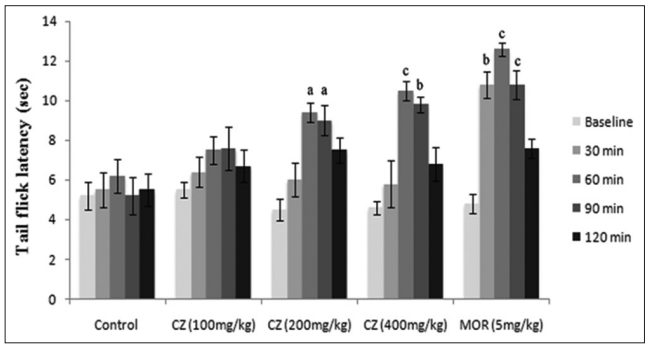

Fig. 2: Effects of hydroalcoholic *Cinnamomum Zeylanicum* (CZ) bark extract on tail flick latency in mice. Mice (n=6 in each group) were pretreated with normal saline, CZ extract (100, 200, and 400 mg/kg, per orally). Morphine (5 mg/kg, i.p) was used as a positive control. TFL was measured 30, 60, 90, and 120 min after administration of drugs. The results are presented as Mean±SEM. Statistical analysis was done by ANOVA followed by *post hoc* Bonferroni test. [a]p<0.05, [b]p<0.001, and [c]p<0.001 as compared to the control group

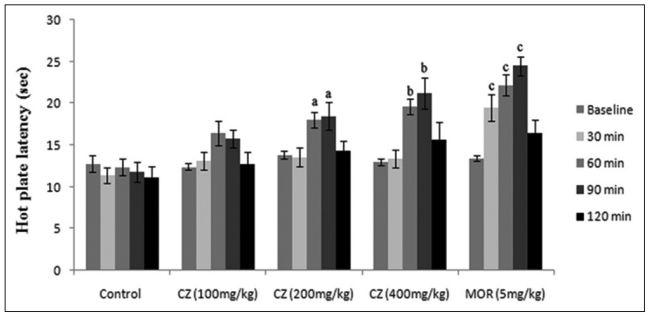

Fig. 3: Effects of hydroalcoholic *Cinnamomum Zeylanicum* (CZ) bark extract in the hot plate test in mice. Mice (n=6 in each group) were pretreated with normal saline, CZ extract (100, 200, and 400 mg/kg, per orally). Morphine (5mg/kg, i.p) was used as a positive control. Latency to reaction time was measured 30, 60, 90, and 120 min after the administration of drugs. The results are presented as Mean ± SEM. Statistical analysis was done by ANOVA followed by *post hoc* Bonferroni test. [a]p<0.05, [b]p<0.001, and [c]p<0.001 as compared to the control group

for culinary purposes. The oil obtained from its leaves, roots, bark, and flowers is mainly used as flavoring agent in astringent powders,

Table 1: Effects of bark extract of Cinnamomum *Zeylanicum* (CZ) on locomotor activity and muscle coordination in mice

Groups	Actophotometer	Fall off time on Rota Rod	
		Before	After
Control	391.6±23.4	56.7±7.2	63.4±7.4
CZ extract (100mg/kg)	496.2±35.6	70.2±7.6	72.5±6.7
CZ extract (200mg/kg)	489.6±46.4	78.5±4.2	74.4±4.9
CZ extract (400mg/kg)	406.2±40.0	80.34±5.9	83.5±6.6

Results are presented as Mean±SEM. Statistical analysis was done by ANOVA followed by *post hoc* Bonferroni test

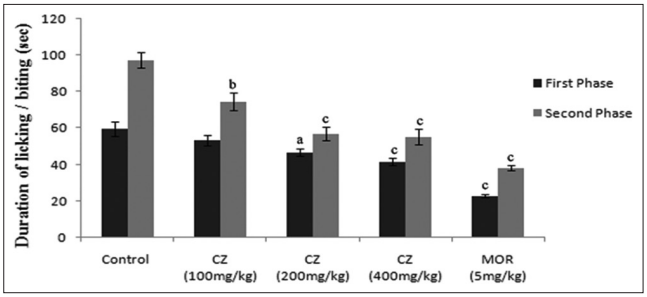

Fig. 4: Effects of hydroalcoholic *Cinnamomum Zeylanicum* (CZ) bark extract on the first and second phase of formalin-induced pain response in mice. Mice (n=6 in each group) were pretreated with normal saline, CZ extract (100, 200, and 400 mg/kg, per orally) I h before the test. Morphine (MOR) (5mg/kg, i.p.) as the positive control was administered 30 min before the test. The results are presented as Mean ± SEM. Statistical analysis was done by ANOVA followed by *post hoc* Bonferroni test. [a]p<0.05, [b]p<0.001, and [c]p<0.001 as compared to the control group

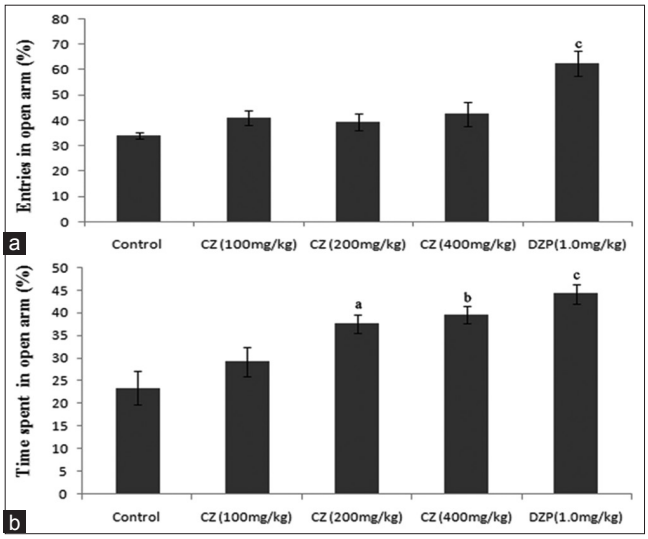

Fig. 5: (a) Effects of hydroalcoholic *Cinnamomum Zeylanicum* (CZ) bark extract on the percentage of open arm entries on elevated plus maze. (b) Effects of hydroalcoholic *Cinnamomum Zeylanicum* (CZ) bark extract on the percentage of time spent in open arm on elevated plus maze. Mice (n=6 in each group) were treated with CMC in distilled water and CZ extract (100, 200, and 400 mg/kg, per orally) 1 h before the test. Diazepam (1mg/kg, i.p.) as the positive control was administered 30 min before the test. The results are presented as Mean ± SEM. Statistical analysis was done by ANOVA followed by *post hoc* Bonferroni test. [a]p<0.05, [b]p<0.001, and [c]p<0.001 as compared to the control group

as antiseptic, and as aromatic in traditional medicine [34]. Its bark is also used in Ayurvedic and Traditional Chinese medicine from ancient time for its antihyperglycemic, digestive, antispasmodic, and antiseptic properties [15,35]. The bark of cinnamon has demonstrated significant antiallergic, antiulcerogenic, antipyretic, and antioxidant activities in experimental studies [7,33,36]. Animal studies have demonstrated that cinnamon and its active constituent cinnamaldehyde, dose-dependently improved glycaemic control and hyperlipidemia in normal and streptozotocin-induced diabetic rats [37]. In a study carried out in the fructose-fed rat, administration of cinnamon bark has been found to improve glucose metabolism and lipid profile through stimulation of antioxidant enzymes [38]. In addition, administration of cinnamon extract has been found to inhibit development and progression of intestinal colitis by inhibiting expression of COX and pro-inflammatory cytokines (IL-1β, TNF-α, and interferon-γ,), suggesting its anti-inflammatory activity [39].

In the present study, the antinociceptive effect of CZ extract was investigated on three experimental models of pain, utilizing thermal (tail flick test and hot plate test) and chemical stimuli (formalin-induced pain response test) to induce pain. Tail flick and hot plate test are used to explore those analgesic compounds that produce antinociceptive effect through centrally mediated mechanisms, while formalin test is used to elucidate those compounds that produce antinociceptive effects through both peripheral and central mechanisms. In another way, it can be implicit that both tail flick and hot plate tests are selectively used to screen the central analgesic agents (e.g., opiates) acting through opioid receptors [40,41].

Tail flick response and hot plate response are believed to involve spinal and supraspinal component, respectively [42]. The results of our study revealed that pretreatment with CZ bark extract significantly increased the TFL and reaction time in hot plate test. These results suggest that CZ extract may possess a centrally mediated antinociceptive effect. Earlier studies [20-23] have reported the antinociceptive effect of cinnamon in the hot plate and acetic acid-induced writhing test. Thus, the results of our study on hot plate test are in accordance with these previous studies and confirm the central antinociceptive effect of CZ bark extract.

Formalin test is commonly used as an animal model of acute inflammatory pain and the nociceptive response induced due to injection of formalin is characterized by two different phases, first and second phases. The first phase (0–5 min) corresponds to acute neurogenic pain or non-inflammatory pain, and it occurs due to direct stimulation of nociceptive myelinated and nonmyelinated sensory fibers by formalin. This phase can be attenuated by centrally acting analgesic drugs acting through the opioidergic pathway. The second phase (30–35 min) of formalin test corresponds to inflammatory pain. During this phase, several inflammatory mediators and cytokines (histamine, serotonin, bradykinins, and prostaglandins) are released in the periphery and spinal cord leading to activation of the neuron. Drugs acting on the central nervous system to reduce pain response can inhibit both phases of formalin-induced nociceptive response while drugs acting in the periphery such as COX enzyme inhibitors and corticosteroids can inhibit only the second phase [43,44].

Results of our study revealed that CZ bark extract abolished formalin-induced pain response in both phases. In the first phase, no effect was seen in 100 mg/kg dose of CZ extract whereas in the dose of 200 and 400 mg/kg significant inhibitory effect was observed on pain response in the first phase. However, the second phase of formalin-induced pain response significantly suppressed with 100, 200, and 400 mg/kg dose of CZ extract. The antinociceptive effect observed in both phases with CZ bark extract indicates the involvement of both peripheral and central mediated mechanisms.

Role of various pro-inflammatory mediators such as interleukin -6 and - 1β (IL-6 and IL-1β), TNF and COX enzymes are well known in the nociceptive process. Literature has shown that administration of cinnamon caused the reduction in the levels of these mediators in various *in vivo* studies, suggesting the anti-inflammatory effect of cinnamon The anti-inflammatory effect of cinnamon reported in these studies is ascribed to trans-cinnamaldehyde component of

cinnamon [39,45,46]. Thus, the antinociceptive effect observed in our study in the formalin test may be due to the reduction in the release of inflammatory mediators following administration of the extract.

In addition, phytochemical screening of extract revealed the presence of several active principles such as phenolic compounds flavonoids and tannins, saponins in the extract and sugars. Thus, the antinociceptive effect observed in the present study might be due to the presence of active constituents also. Many investigators have found that phenolic compounds, flavonoids, and tannins are able to inhibit metabolism of arachidonic acid, release of histamine from mast cells, and platelet aggregation and showed antinociceptive, anti-inflammatory and antioxidant activity in *in vivo* and *in vitro* studies [23,47] It is reported that saponins bind on sensory nerve terminals and have shown analgesic effects through opioid receptor mechanism [48]. Hence, the presence of the phenolic compound, cinnamaldehyde, flavonoids, and others may contribute to the antinociceptive activity of CZ bark extract. Besides, investigators have also reported the potent antioxidant action of cinnamon in various experimental studies. There are reports which indicate the contribution of increased generation of reactive oxygen species in formalin-induced nociception [49]. Hence, it can be assumed that the antinociceptive effect of CZ bark extract may be due to both anti-inflammatory and antioxidant properties. Rotarod test and actophotometer tests were also performed to rule out any effect of the locomotor deficit on observed pain response. No change in locomotor activity or muscle coordination was observed following administration of CZ bark extract.

Results of elevated plus maze tests showed a significant increase in the time spent in the open arms at 200 and 400 mg/kg doses of CZ bark when compared to the control group. Several studies have reported the association of increased oxidative stress and inflammation with anxiety disorders [50,51]. Thus, the anxiolytic effect observed in this study may be attributed to the anti-inflammatory and antioxidant action of cinnamon. Previous experimental evidence has shown the anxiolytic effect of cinnamon and our findings on anxiety-like behavior are in concurrence with the observation of these studies [52,53]. In one study, extract of *Cinnamomum cassia* has been shown to exert anxiolytic effects through regulation of serotonin and gamma-Aminobutyric acid (GABA) pathways [54] hence, it can be assumed that extract of CZ bark in the present study might also produce anxiolytic effect through modulation of these pathways. Thus, it can be concluded that the administration of CZ extract showed inhibitory effect on both heat and chemical-induced pain, indicating that CZ extract exerts its antinociceptive effect through both peripheral and central action. In addition, the anxiolytic effect was also observed. However, more detailed studies are warranted in this direction to decode the exact mechanism responsible for the antinociceptive and anxiolytic effect of cinnamon.

ACKNOWLEDGMENTS

The authors would like to thanks the Advanced Instrumentation Research Facility, Jawaharlal Nehru University, India, for conducting the GC-MS analysis of extract.

AUTHORS' CONTRIBUTIONS

Both authors have contributed equally to the study. The first author mainly contributed to the study design, ethical approval of the study, writing of the manuscript, and performed experimental procedures in different groups included in the study. The second author was mainly involved in the analysis of data and writing of the manuscript.

CONFLICTS OF INTEREST

The authors state that there are no conflicts of interest regarding the publication of this article.

REFERENCES

1. Bbalijepalli MK, Buru AS, Sakirolla R, Pichika MR. *Cinnamomum* genus: A review on its biological activities. Int J Pharm Pharm Sci 2017;9:1-11.
2. Aneja K, Joshi R, Sharma C. Antimicrobial activity of dalchini (*Cinnamomum zeylanicum* bark) extracts on some dental caries pathogens. J Pharm Res 2009;2:1387-90.
3. Chao LK, Hua KF, Hsu HY, Cheng SS, Liu JY, Chang ST. Study on the antiinflammatory activity of essential oil from leaves of *Cinnamomum osmophloeum*. J Agric Food Chem 2005;53:7274-8.
4. Ravindran PN, Nirmal-Babu K, Shylaja M. Cinnamon and Cassia: The genus *Cinnamomum*. Danvers, MA: CRC Press; 2003.
5. López P, Sánchez C, Batlle R, Nerín C. Solid- and vapor-phase antimicrobial activities of six essential oils: Susceptibility of selected foodborne bacterial and fungal strains. J Agric Food Chem 2005;53:6939-46.
6. Dongmo PM, Tatsadjieu LN, Tchoumbougnang F, Sameza ML, Ndongson B, Zollo PH, *et al*. Chemical composition, antiradical and antifungal activities of essential oil of the leaves of *Cinnamomum zeylanicum* Blume from Cameroon. Nat Prod Commun 2007;12:1287-90.
7. Kurokawa M, Kumeda CA, Yamamura J, Kamiyama T, Shiraki K. Antipyretic activity of cinnamyl derivatives and related compounds in influenza virus-infected mice. Eur J Pharmacol 1998;348:45-51.
8. Khan A, Safdar M, Ali Khan MM, Khattak KN, Anderson RA. Cinnamon improves glucose and lipids of people with Type 2 diabetes. Diabetes Care 2003;26:3215-8.
9. Lee JS, Jeon SM, Park EM, Huh TL, Kwon OS, Lee MK, *et al.* Cinnamate supplementation enhances hepatic lipid metabolism and antioxidant defense systems in high cholesterol-fed rats. J Med Food 2003;6:183-91.
10. Jayaprakasha GK, Ohnishi-Kameyama M, Ono H, Yoshida M, Jaganmohan Rao L. Phenolic constituents in the fruits of *Cinnamomum zeylanicum* and their antioxidant activity. J Agric Food Chem 2006;54:1672-9.
11. Soliman KM, Badeaa RI. Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi. Food Chem Toxicol 2002;40:1669-75.
12. Anderson RA, Broadhurst CL, Polansky MM, Schmidt WF, Khan A, Flanagan VP, *et al.* Isolation and characterization of polyphenol Type-A polymers from cinnamon with insulin-like biological activity. J Agric Food Chem 2004;52:65-70.
13. Jain S, Sangma T, Shukla SK, Mediratta PK. Effect of *Cinnamomum zeylanicum* extract on scopolamine-induced cognitive impairment and oxidative stress in rats. Nutr Neurosci 2015;18:210-6.
14. Shaughnessy DT, Setzer RW, DeMarini DM. The antimutagenic effect of vanillin and cinnamaldehyde on spontaneous mutation in salmonella TA104 is due to a reduction in mutations at GC but not AT sites. Mutat Res 2001;480-481:55-69.
15. Stavinoha RC, Vattem DA. Potential neuroprotective effects of cinnamon. Int J Appl Res Nat Prod 2015;8:24-46.
16. Tomaino A, Cimino F, Zimbalatti V, Venuti V, Sulfaro V, De Pasquale V, *et al*. Influence of heating on antioxidant activity and the chemical composition of some spice essential oils. Food Chem 2005;89:549-54.
17. Lee SH, Lee SY, Son DJ, Lee H, Yoo HS, Song S, *et al.* Inhibitory effect of 2'-hydroxycinnamaldehyde on nitric oxide production through inhibition of NF-kappa B activation in RAW 264.7 cells. Biochem Pharmacol 2005;69:791-9.
18. Fu Y, Yang P, Zhao Y, Zhang L, Zhang Z, Dong X, *et al. Trans*-cinnamaldehyde inhibits microglial activation and improves neuronal survival against neuroinflammation in BV2 microglial cells with lipopolysaccharide stimulation. Evid Based Complement Alternat Med 2017;2017:4730878.
19. Joshi K, Awte S, Bhatnagar P, Walunj S, Gupta R, Joshi S, *et al*. *Cinnamomum zeylanicum* extract inhibits proinflammatory cytokine TNF: *In vitro* and *in vivo* studies. Res Pharm Biotech 2010;2:14-21.
20. Dashti-R MH, Qane MD, Shefaie F, Yazdu MN, Bagheri SM. Comparative effect of cinnamon essential oil, diclofenac and morphine on acute and chronic pain in mice. Int J Med Lab 2016;3:92-103.
21. Churihar R, Solanki P, Vyas S, Tanwani H, Atal S. Analgesic activity of cinnamaldehyde per se and it's interaction with diclofenac sodium and pentazocine in Swiss albino mice. Int J Phamacol 2016;3:97-102.
22. Izadpanah E, Nikandam F, Moloudi MR, Hassanzadeh K. Evaluation of the analgesic effect of hydroalcoholic extract of *Cinnamomum* in rats. Sci J Kurdistan Univ Med Sci 2016;21:1-8.
23. Atta AH, Alkofahi A. Anti-nociceptive and anti-inflammatory effects of some Jordanian medicinal plant extracts. J Ethnopharmacol 1998;60:117-24.
24. Trease EG, Evans WC. Textbook of Pharmacognosy. 12th ed. Singapore: Alden Press; 1983. p. 539-41.
25. D'Amour FE, Smith DL. A method for determining loss of pain

sensation. J Pharmacol Exp Ther 1941;72:74-9.
26. Pires JM, Mendes FR, Negri G, Duarte-Almeida JM, Carlini EA. Antinociceptive peripheral effect of *Achillea millefolium* L. And *Artemisia vulgaris* L.: Both plants known popularly by brand names of analgesic drugs. Phytother Res 2009;23:212-9.
27. Abbott FV, Franklin KB, Westbrook RF. The formalin test: Scoring properties of the first and second phases of the pain response in rats. Pain 1995;60:91-102.
28. Kulkarni SK, Dandiya PC. Influence of intraventricular administration of norepinephrine, dopamine and 5-hydroxytryptamine on motor activity of rats. Indian J Med Res 1975;63:462-8.
29. Bharal N, Sahaya K, Jain S, Mediratta PK, Sharma KK. Curcumin has anticonvulsant activity on increasing current electroshock seizures in mice. Phytother Res 2008;22:1660-4.
30. Abdel-Salam OM, Baiuomy AR, Nada SA. Effect of spironolactone on pain responses in mice. EXCLI J 2010;9:46-57.
31. Dunham NW, Miya TS. A note on a simple apparatus for detecting neurological deficit in rats and mice. J Am Pharm Assoc Am Pharm Assoc 1957;46:208-9.
32. Pellow S, Chopin P, File SE, Briley M. Validation of open: Closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 1985;14:149-67.
33. Torizuka K. Basic lecture of kampo medicine: Pharmacological effect of cinnamon. Kampo Med 1998;11:431-6.
34. Sachan AK, Kumar S, Kumari K, Singh D. Medicinal uses of spices used in our traditional culture: Worldwide. J Med Plants 2018;6:116-22.
35. AL-Logmani AS, Zari TA. Effects of *Nigella sativa* L. and *Cinnamomum zeylanicum* Blume oils on some physiological parameters in streptozotocin-induced diabetic rats. Bol Latinoam Caribe Plantas Med Aromát 2009;8:86-96.
36. Dhuley JN. Anti-oxidant effects of cinnamon (*Cinnamomum verum*) bark and greater cardamom (*Amomum subulatum*) seeds in rats fed high fat diet. Indian J Exp Biol 1999;37:238-42.
37. Kim SH, Hyun SH, Choung SY. Anti-diabetic effect of cinnamon extract on blood glucose in db/db mice. J Ethnopharmacol 2006;104:119-23.
38. Kannappan S, Jayaraman T, Rajasekar P, Ravichandran MK, Anuradha CV. Cinnamon bark extract improves glucose metabolism and lipid profile in the fructose-fed rat. Singapore Med J 2006;47:858-63.
39. Kwon HK, Hwang JS, Lee CG, So JS, Sahoo A, Im CR, *et al.* Cinnamon extract suppresses experimental colitis through modulation of antigen-presenting cells. World J Gastroenterol 2011;17:976-86.
40. Hasan SR, Mariam J, Majumder MM, Raushanara A, Hossain MM, Mazumder M, *et al*. Analgesic and antioxidant activity of the hydromethanolic extract of *Mikania scandens* (L.) Willd. Leaves. Am J Pharmacol Toxicol 2009;4:1-7.
41. Mansouri MT, Naghizadeh B, Ghorbanzadeh B. Sildenafil enhances the peripheral antinociceptive effect of ellagic acid in the rat formalin test. Indian J Pharmacol 2014;46:404-8.
42. Sinclair JG, Main CD, Lo GF. Spinal vs. Supraspinal actions of morphine on the rat tail-flick reflex. Pain 1988;33:357-62.
43. Sridharan S, Venkatramani M, Janakiraman K, Pemiah B, Chinnagounder S. Barleria Montana wight and Nees a promising natural anti-inflammatory agent against formalin induced inflammation. Int J Pharm Pharm Sci 2015;7:80-4.
44. Shibata M, Ohkubo T, Takahashi H, Inoki R. Modified formalin test: Characteristic biphasic pain response. Pain 1989;38:347-52.
45. Song F, Li H, Sun J, Wang S. Protective effects of cinnamic acid and cinnamic aldehyde on isoproterenol-induced acute myocardial ischemia in rats. J Ethnopharmacol 2013;150:125-30.
46. Chao LK, Hua KF, Hsu HY, Cheng SS, Lin IF, Chen CJ, *et al.* Cinnamaldehyde inhibits pro-inflammatory cytokines secretion from monocytes/macrophages through suppression of intracellular signaling. Food Chem Toxicol 2008;46:220-31.
47. Zeashan H, Amresh G, Rao CV, Singh S. Antinociceptive activity of *Amaranthus spinosus* in experimental animals. J Ethnopharmacol 2009;122:492-6.
48. Nguyen TT, Matsumoto K, Yamasaki K, Nguyen MD, Nguyen TN, Watanabe H, *et al.* Crude saponin extracted from vietnamese ginseng and its major constituent majonoside-R2 attenuate the psychological stress- and foot-shock stress-induced antinociception in mice. Pharmacol Biochem Behav 1995;52:427-32.
49. Hacimuftuoglu A, Handy CR, Goettl VM, Lin CG, Dane S, Stephens RL Jr., *et al.* Antioxidants attenuate multiple phases of formalin-induced nociceptive response in mice. Behav Brain Res 2006;173:211-6.
50. Rawdin BJ, Mellon SH, Dhabhar FS, Epel ES, Puterman E, Su Y, *et al.* Dysregulated relationship of inflammation and oxidative stress in major depression. Brain Behav Immun 2013;31:143-52.
51. Salim S. Oxidative stress and psychological disorders. Curr Neuropharmacol 2014;12:140-7.
52. Sohrabi R, Pazgoohan N, Seresht HR, Amin B. Repeated systemic administration of the cinnamon essential oil possesses anti-anxiety and anti-depressant activities in mice. Iran J Basic Med Sci 2017;20:708-14.
53. Fadaei S. Asle-Rousta M. Anxiolytic and antidepressant effects of cinnamon (*Cinnamomum verum*) extract in rats receiving lead acetate. Sci J K Univ 2018;22:31-9.
54. Yu HS, Lee SY, Jang CG. Involvement of 5-HT1A and GABAA receptors in the anxiolytic-like effects of *Cinnamomum cassia* in mice. Pharmacol Biochem Behav 2007;87:164-70.